

Figure 1

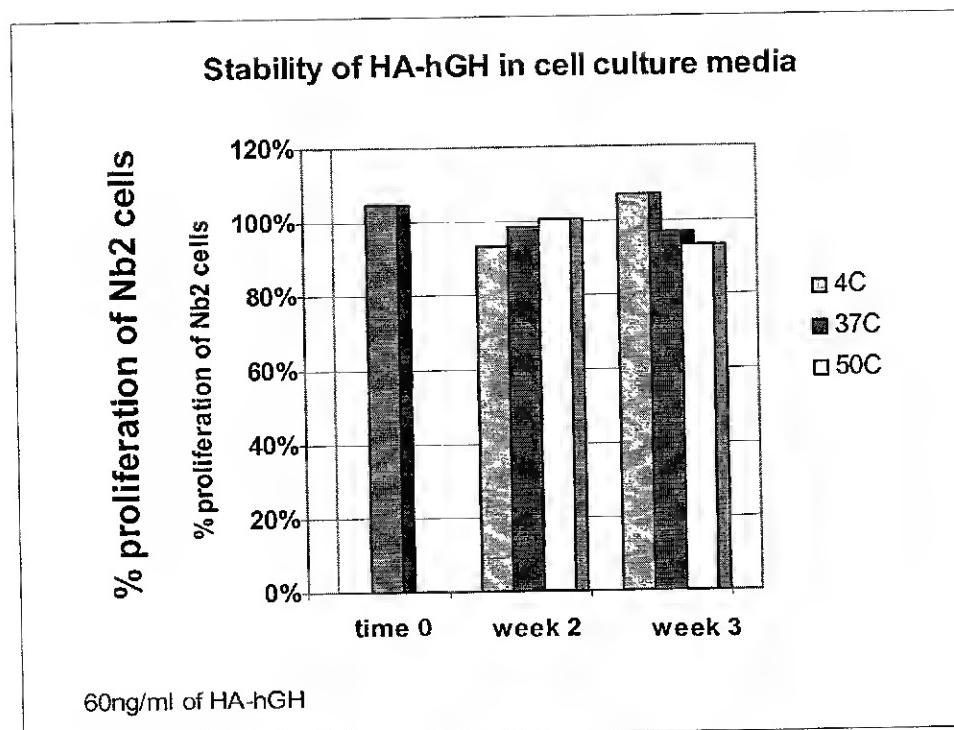


Figure 2

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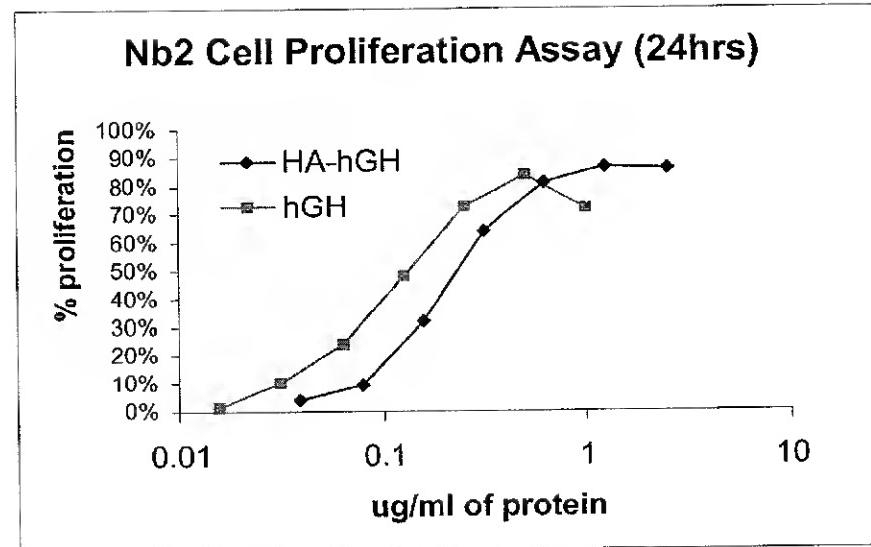


Figure 3A

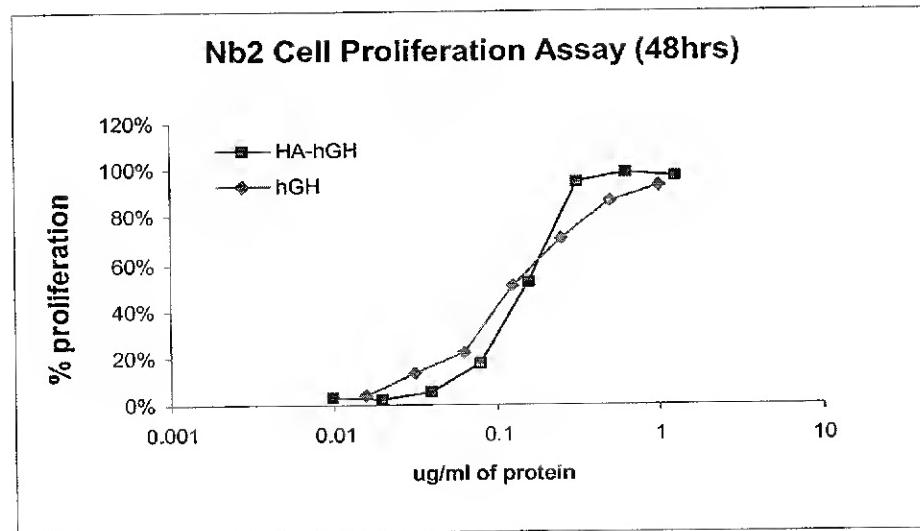


Figure 3B

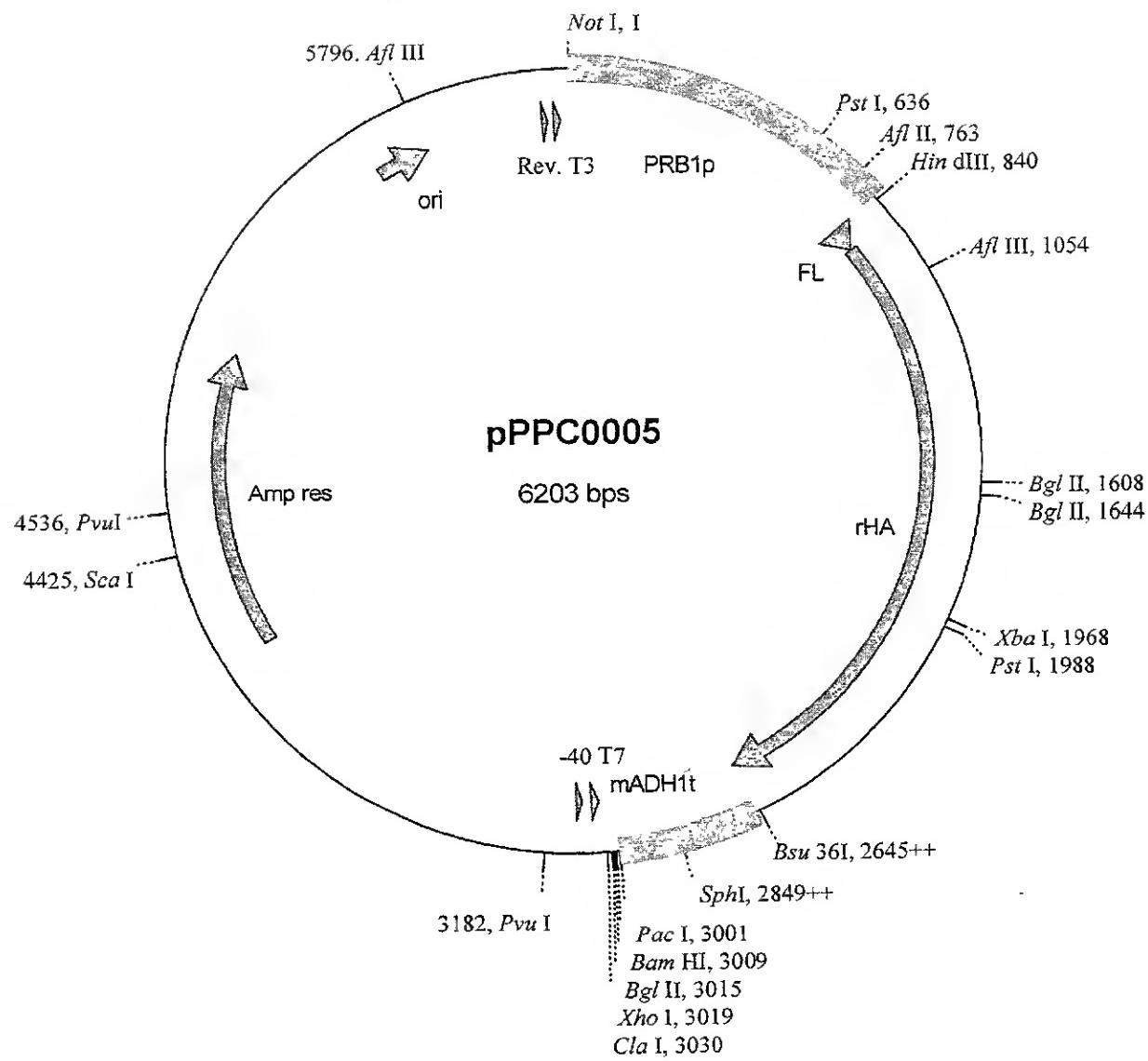


Figure 4

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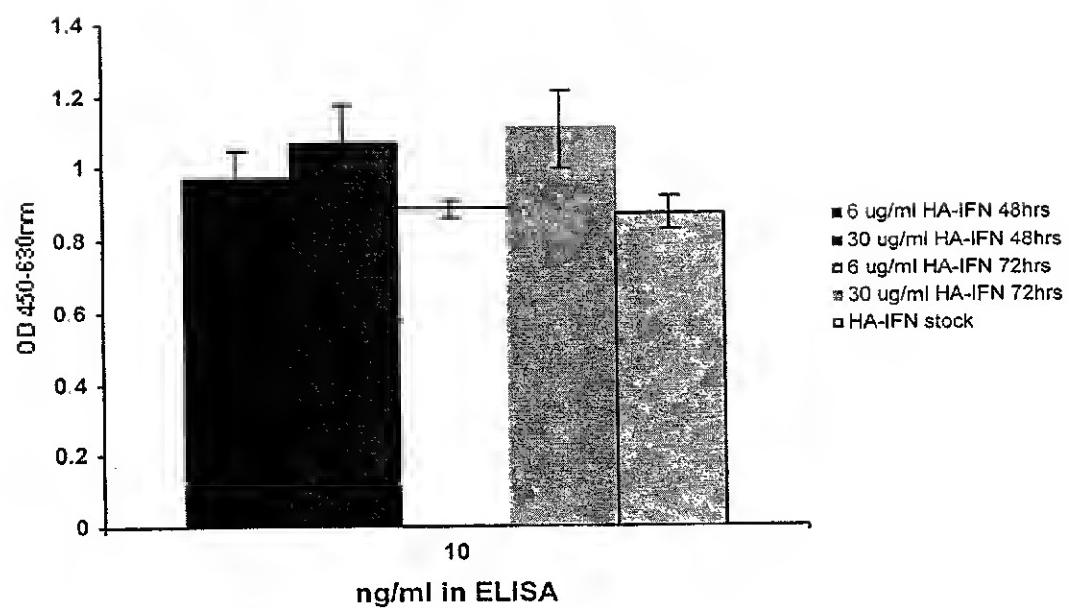
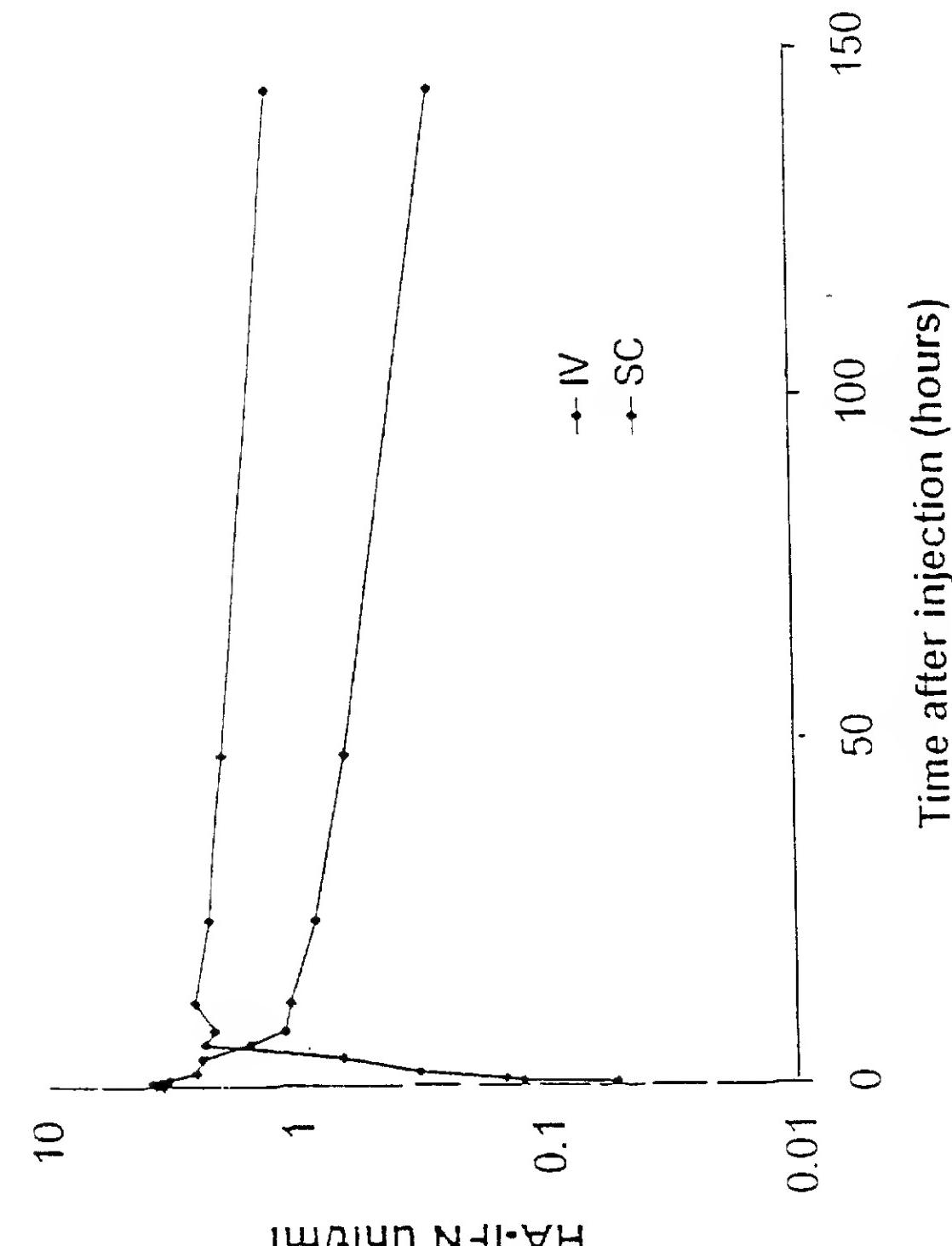


Figure 5

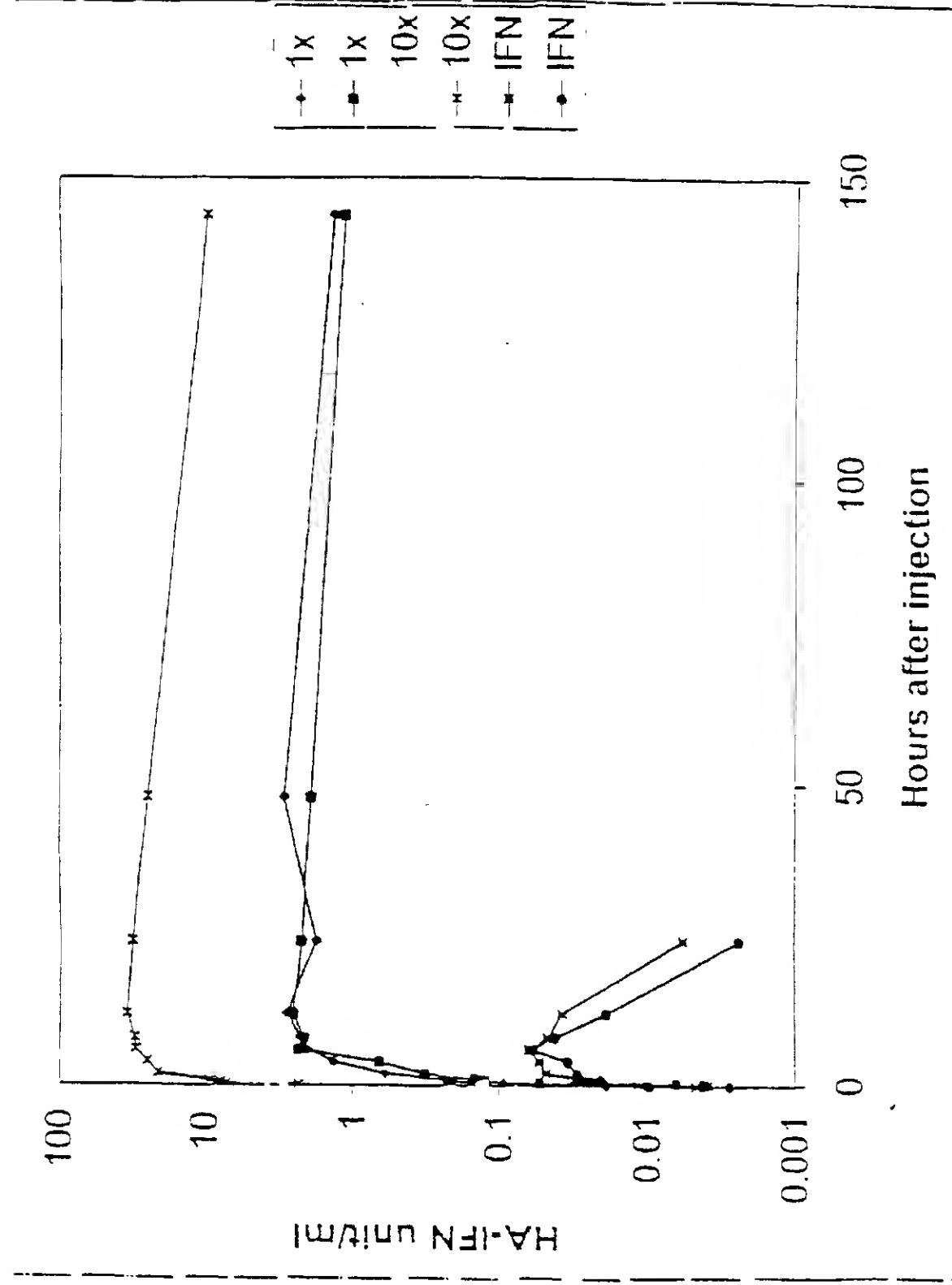
SCANNED, # 6

Figure 6



SCANNED, # 6

Figure 7



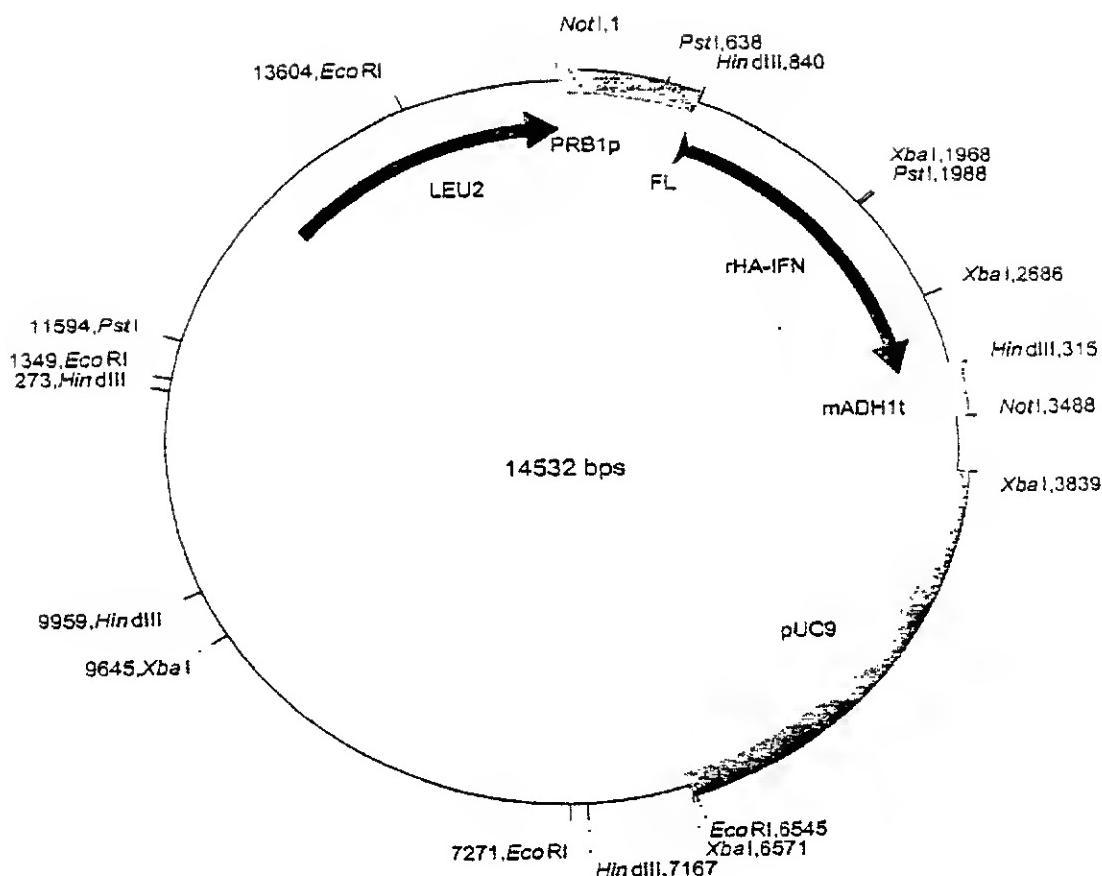


Figure 8. The HA-IFN α expression cassette in pSAC35. The expression cassette comprises
PRB1 promoter, from *S. cerevisiae*.
 Fusion leader, first 19 amino acids of the HA leader followed by the last 6 amino acids of the MFA-1 leader.
 HA-IFN α coding sequence with a double stop codon (TAATAA)
ADH1 terminator, from *S. cerevisiae*. Modified to remove all the coding sequence normally present in the *Hind* III/*Bam* HI fragment generally used.

Figure 8

Localisation of ‘Loops’ based on the HA Crystal Structure which could be used for Mutation/Insertion

1 DAHKSEVAHR FKDLGEENFK ALVLIAFAQY LQQCPFEDHV KLVNEVTEFA
HHHHH HHH HHH HHHHHHHHHHH HHHHH HHHHHHHHHHH

I II III

51 KTCVADESAE NCDKSLHTLF GDKLCTVATL RETYGEMADC CAKOEPERNE
HHHHH HHHHH HHH H HHHHH

101 CFLQHKDDNP NLPRLVRPEV DVMCTAFHDN EETFLKKYLY EIARRHPYFY
HHHH H HHHHHHHHH HHHHHHHHH HHHHH

IV

151 APPELLFFAKR YKAATTECCO AADKAACLLP KLDELRDEGK ASSAKQLKC
HHHHHHHHHH HHHHHHHHH HHHHH HHHEHHHHHH HHHHHHHHHHH

V

201 ASLQKFGERA FKAavarls QRFPKAEEAE VSKLVTDLTK VTecchGDL
HHHHH HH HHHHHHHHH HH HHH HHHHHHHHH HHHHH HH

VI VII

251 LECADDRADL AKYICENODS ISSKLKECCE KPLLEKSHCI AEVENDEMPA
HHHHHHHHHH HHHHH HHHHH HHHHH HHHHH H

301 DLPSLAADFV ESKDVCKNYA EAKDVFLGMF LYEYARRHPD YSVVLLRLA
HHHH HHHHH HHHHHHHHH HHHHH HHHHHHHHH

VIII

351 KTYETTLEKC CAAADPHECY AKVFDEFKPL VEEPQNLIKQ NCELFEQLGE
HHHHHHHHHH HH H HHHHH HHHHHHHHHHH HHHHHHHHH

IX

401 YKFQNALLVR YTkkVPQVST PTLVEVSRLN GKVGSKCCKH PEAKRMPCAE
HHHHHHHHHH HHH H HHHHHHHHH HHH HHHHHHHHH

X XI

451 DYLSVVLMQL CVLHEKTPVS DRVTKCCTES LVNRRPPCFSA LEVDETYVPK
HHHHHHHHHH HHHHH HHHHHHHHH HHHHHHHHH

501 EFNAETFTFH ADICTLSEKE RQIKKQTALV ELVKHKPKAT KEQLKAVMDD
HHH HHH HHHHMMEHHH HHH HHHHHHHHH

XII

551 FAAFVEKCCK ADDKETCFAE EGKKLVAASQ AALGL
HHHHHHHH HHHHHHHHHHH HHH

Loop		Loop	
I	Val154-Asn61	VII	Glu280-His288
II	Thr76-Asp89	VIII	Ala362-Glu368
III	Ala92-Glu100	IX	Lys439-Pro447
IV	Gln170-Ala176	X	Val462-Lys475
V	His247-Glu252	XI	Thr478-Pro486
VI	Glu266-Glu277	XII	Lys560-Thr566

Figure 9

Examples of Modifications to Loop IV

a. Randomisation of Loop IV.

IV
151 APELLFFAKR YKA~~A~~FTTECCO AADKAACLLP KLDDELRDEGK ASSAKQRLKC
HHHHHHHHHHHH HHHHHHHHHHH HHHHHHHHHHHHH HHHHHHHHHHHHH

151 APELLFFAKR YKAAFTTECCX XXXXXXCLLP KLDELRDEGK ASSAKQRLKC
HHHHHHHHHHH HHHHHHHHHH HHHHH HHHHHHHHHHHH HHHHHHHHHHH

X represents the mutation of the natural amino acid to any other amino acid. One, more or all of the amino acids can be changed in this manner. This figure indicates all the residues have been changed.

b. Insertion (or replacement) of Randomised sequence into Loop IV.

(X)_n
↓
IV
151 APPELLFFAKR YKAAFTECCQ AADKAACLLP KLDELRDEGK ASSAKQLKCA
HHHHHHHHHHHHH HHHHHHHHHH HHHHHHHHHHHH HHHHHHHHHH

Figure 10

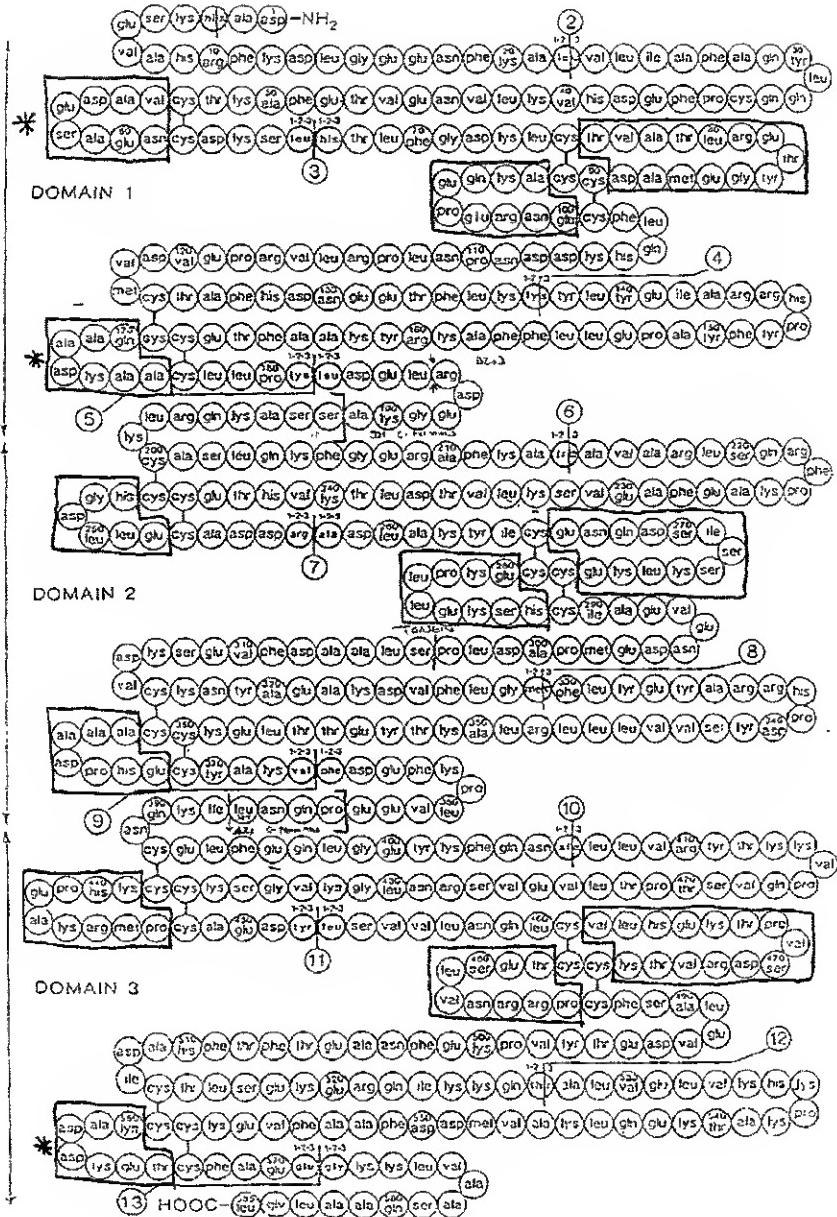
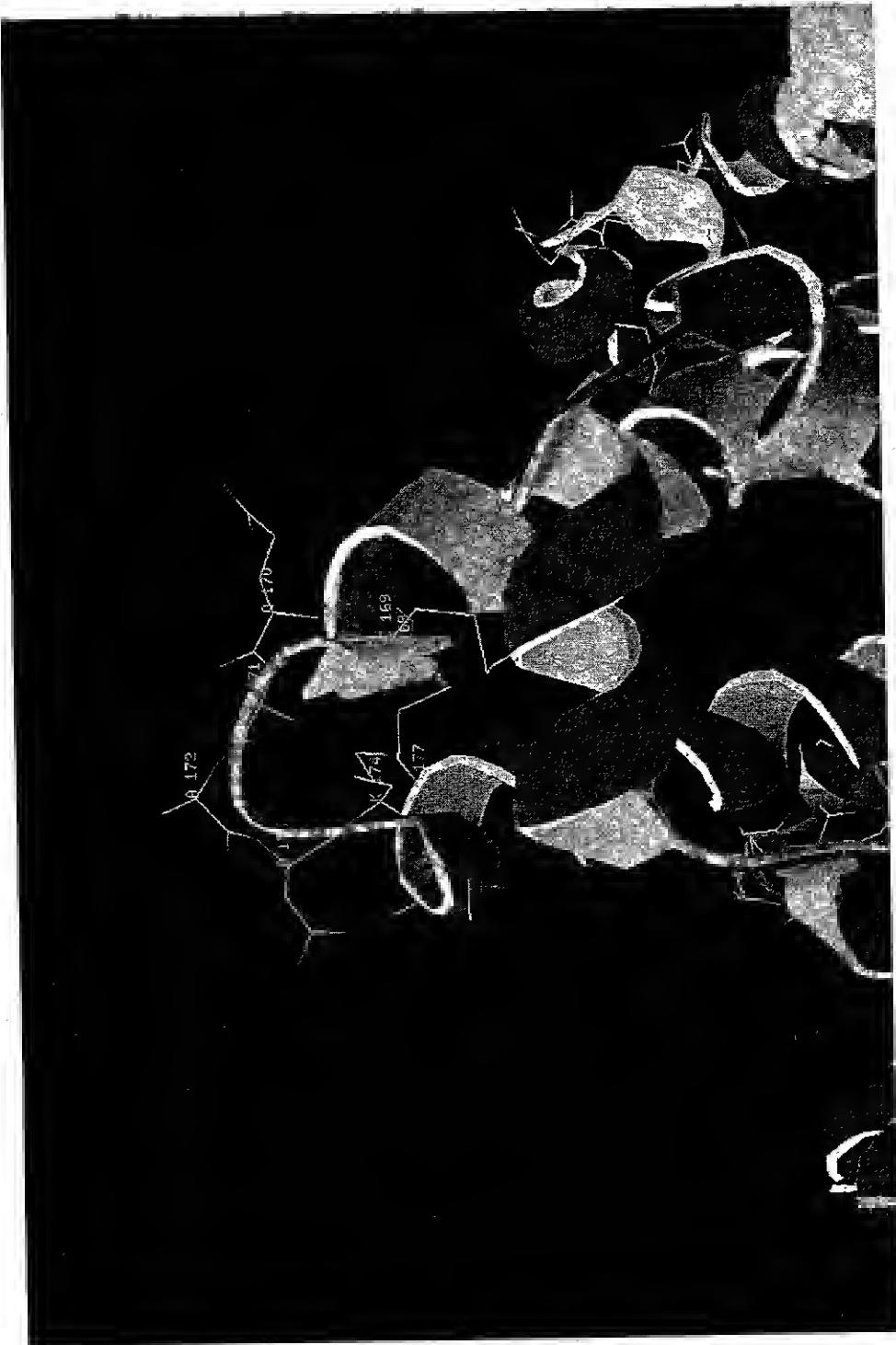


Figure 11



Disulfide bonds shown in yellow

Figure 12: Loop IV Gln170-Ala176

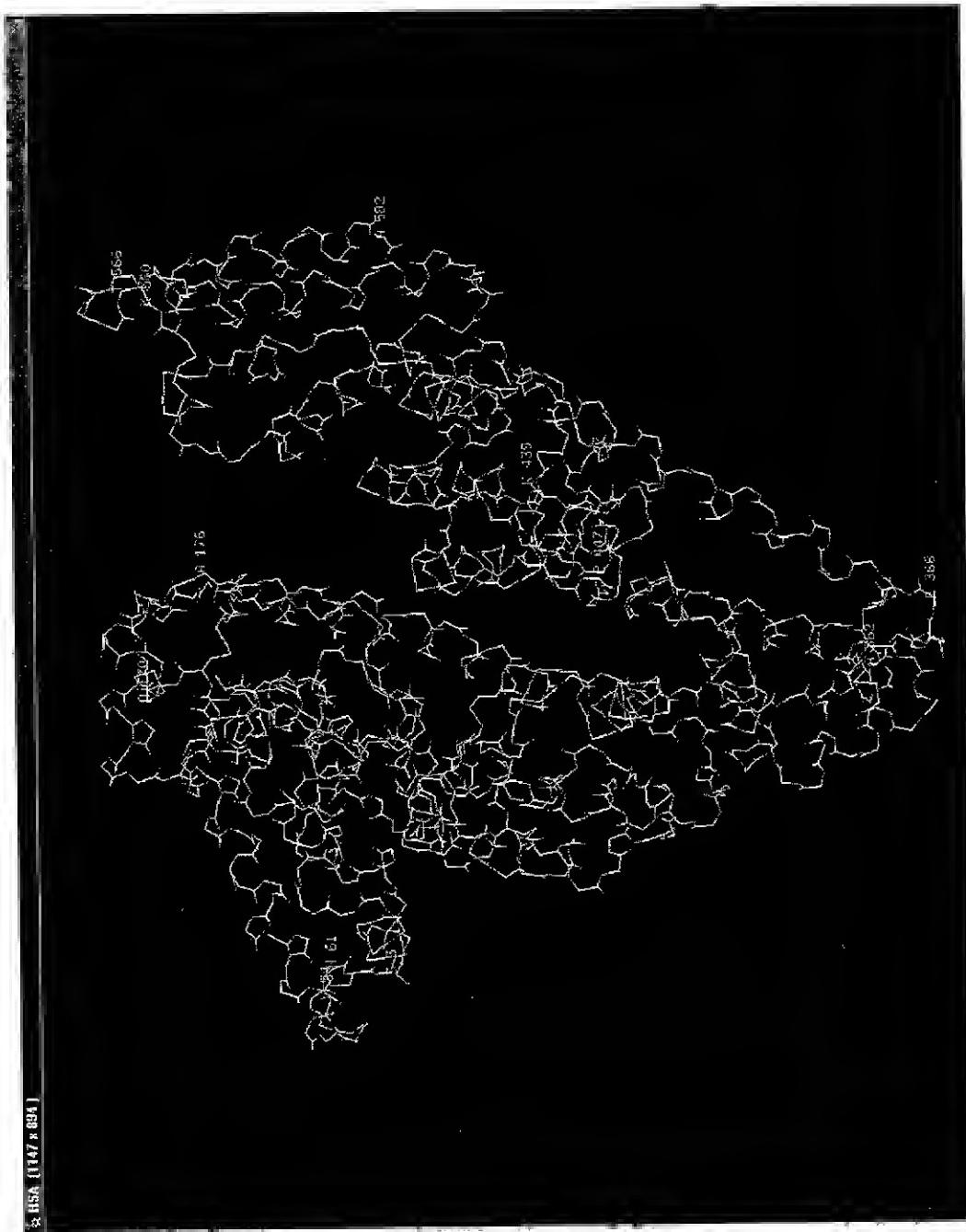
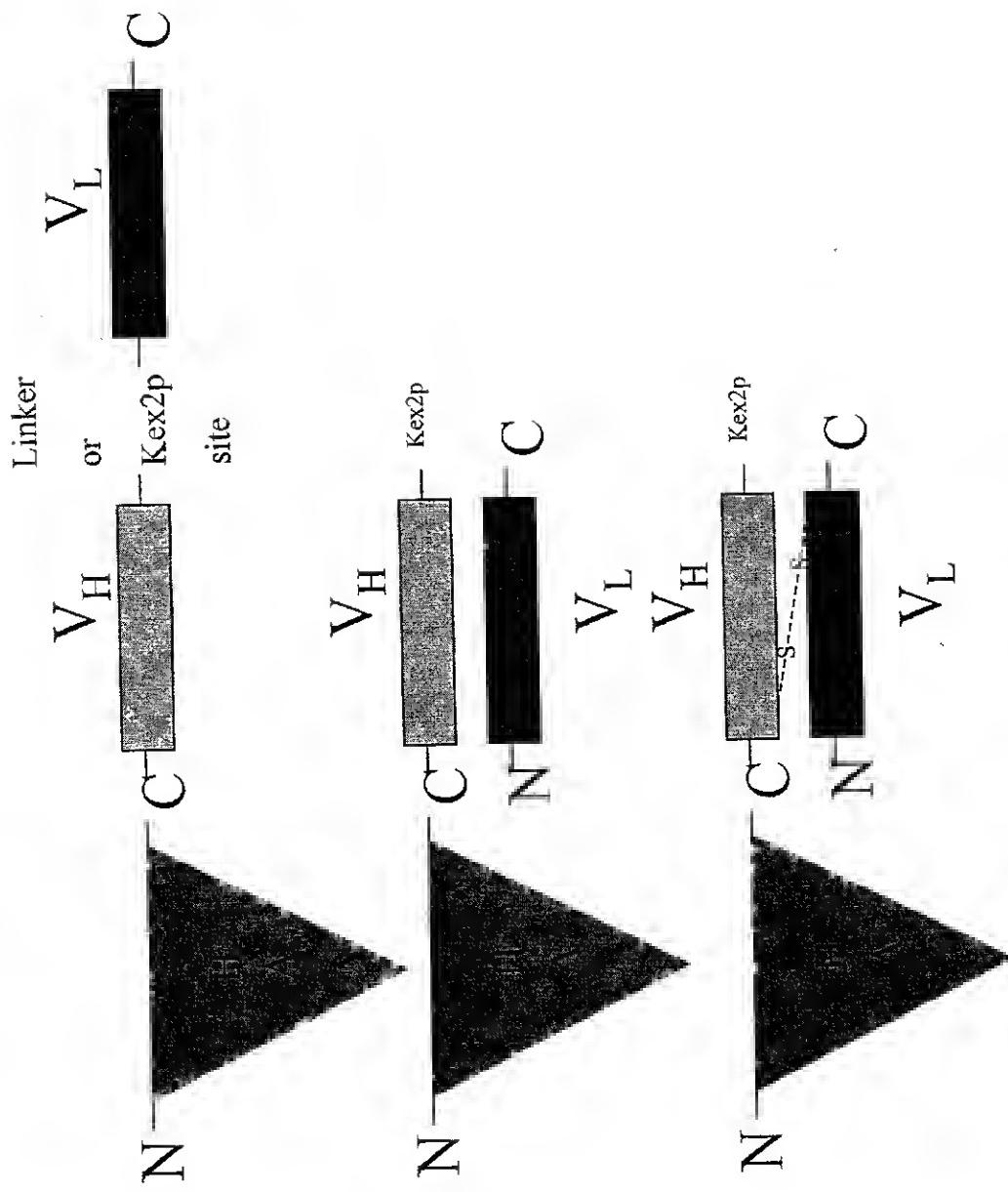


Figure 13: Tertiary Structure of HA



**Figure 14: Schematic Diagram of Possible ScFv Fusions
(Example is of a C-terminal fusion to HA)**

15/18 GTGCTTGTGAGCTTGCGATGAA

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1 GAT GCA CAC AAG AGT GAG GCT CAT CGG TTT AAA GAT TTG GGA GAA GAA AAT TTC AAA 60
1 D A H K S E V A H R F K D L G E E N F K 20

61 GCC TTG GTG ATT GCC TTT GCT CAG TAT CTT CAG CAG TGT CCA TTT GAA GAT CAT GTA 120
21 A L V L I A F A Q Y L Q Q C P F E D H V 40

121 AAA TTA GTG AAT GAA GTA ACT GAA TTT GCA AAA ACA TGT GTT GCT GAT GAG TCA GCT GAA 180
41 K L V N E V T E F A K T C V A D E S A E 60

181 AAT TGT GAC AAA TCA CTT CAT ACC CTT TTT GGA GAC AAA TTA TGC ACA GTT GCA ACT CTT 240
61 N C D K S L H T L F G D K L C T V A T L 80

241 CGT GAA ACC TAT GGT GAA ATG GCT GAC TGC TGT GCA AAA CAA GAA CCT GAG AGA AAT GAA 300
81 R E T Y G E M A D C C A K Q E P E R N E 100

301 TGC TTC TTG CAA CAC AAA GAT GAC AAC CCA AAC CTC CCC CGA TTG GTG AGA CCA GAG GTT 360
101 C F L Q H K D D N P N L P R L V R P E V 120

361 GAT GTG ATG TGC ACT GCT TTT CAT GAC AAT GAA GAG ACA TTT TTG AAA AAA TAC TTA TAT 420
121 D V M C T A F H D N E E T F L K Y L Y 140

421 GAA ATT GCC AGA AGA CAT CCT TAC TTT TAT GCC CCG GAA CTC CTT TTC TTT GCT AAA AGG 480
141 E I A R R H P Y F V A P E L L F A K R 160

Figure 15A

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481 TAT AAA GCT GCT TTT ACA GAA TGT TGC CAA GCT GAT AAA GCT GCC TCG CTG TGC TGT CCA 540
161 Y K A A F T E C C Q A A D K A A C L L P 180

541 AAG CTC GAT GAA CTT CGG GAT GAA GGG AAG GCT TCG TCT GCC AAA CAG AGA CTC AAA TGT 600
181 K L D E L R D E G K A S S A K Q R L K C 200

601 GCC AGT CTC CAA AAA TTT GGA GAA AGA GCT TTC AAA GCA TGG GCA GTG GCT CGC CTG AGC 660
201 A S L Q K F G E R A F K A W A V A R L S 220

661 CAG AGA TTT CCC AAA GCT GAG TTT GCA GAA GTT TCC AAG TTA GTG ACA GAT CTT ACC AAA 720
221 Q R F P K A E F A E V S K L V T D L T K 240

721 GTC CAC ACG GAA TGC TGC CAT GGA GAT CTT GAA TGT GCT GAT GAC AGG GCG GAC CTT 780
241 V H T E C C H G D L L E C A D D R A D L 260

781 GCC AAG TAT ATC TGT GAA AAT CAG GAT TCG ATC TCC AGT AAA CTG AAG GAA TGC TGT GAA 840
261 A K Y I C E N Q D S I S K L K E C C E 280

841 AAA CCT CTG TTG GAA AAA TCC CAC TGC ATT GCC GAA GTG GAA AAT GAT GAG ATG CCT GCT 900
281 K P L E K S H C I A E V E N D E M P A 300

901 GAC TTG CCT TCA TTA GCT GAT TTT GTT GAA AGT AAG GAT GTT TGC AAA AAC TAT GCT 960
301 D L P S L A D F V E S K D V C K N Y A 320

Figure 15B

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961 GAG GCA AAG GAT GTC TTC CTG CGC ATG TTT TTG TAT GCA AGA TAT CCT AGG CAT CCT GAT 1020
321 E A K D V F L G M F L Y E Y A R R H P D 340

1021 TAC TCT GTC GTC GTG CTG CTG AGA CTT GCC AAG ACA TAT GAA ACC ACT CTA GAG AAG TGC 1080
341 Y S V V L L R L A K T Y E T T L E K P C 360

1081 TGT GCC GCT GCA GAT CCT CAT GAA TGC TAT GCC AAA GTG TTC GAT GAA TTT AAA CCT CTT 1140
361 C A A D P H E C Y A K V F D E F K P L 380

1141 GTG GAA GAG CCT CAG AAT TTA ATC AAA CAA AAC TGT GAG CTT TTT GAG CAG CTT GGA GAG 1200
381 V E E P Q N L I K Q N C E L F E Q L G E 400

1201 TAC AAA TTC CAG AAT GCG CTA TTA GTT CGT TAC ACC AAG AAA GTA CCC CAA GTG TCA ACT 1260
401 Y K F Q N A L L V R Y T K V P Q V S T 420

1261 CCA ACT CTT GTA GAG GTC TCA AGA AAC CTA GGA AAA GTG GGC AGC AAA TGT TGT AAA CAT 1320
421 P T L V E V S R N L G K V G S K C C K H 440

1321 CCT GAA GCA AAA AGA ATG CCC TGT GCA GAA GAC TAT CTA TCC GTG GTC AAC CAG TTA 1380
441 P E A K R M P C A E D Y L S V V L N Q L 460

1381 TGT GTG TTG CAT GAG AAA ACG CCA GTA AGT GAC AGA GTC ACA AAA TGC TGC ACA GAG TCC 1440
461 C V L H E K T P V S D R V T K C C T E S 480

Figure 15C

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1441 T^rG G^tG AAC AGG CGA CCA TGC T^rT TCA GCT CTG GAA GTC GAT GAA ACA TAC GTT CCC AAA 1500
481 L V N R R P C F S A L E V D E T Y V P K 500

1501 GAG T^rT AAT GCT GAA ACA T^rC ACC T^rC CAT GCA GAT ATA TGC ACA CTT TCT GAG AAG GAG 1560
501 E F N A E T F T F H A D I C T L S E K E 520

1561 AGA CAA ATC AAG AAA CAA ACT GCA CTT GAG CTT GAG AAA CAC AAG CCC AAG GCA ACA 1620
521 R Q I K Q T A L V E L V K H K P K A T 540

1621 AAA GAG CAA CTG AAA GCT G^tT ATG GAT GAT T^rT GCA GCT T^rT GTA GAG AAG TGC TGC AAG 1680
541 K E Q L K A V M D D F A A F V E K C C K 560

1681 GCT GAC GAT AAG GAG ACC TGC T^rT GCC GAG GAG GGT AAA AAA CTT G^tT GCT GCA AGT CAA 1740
561 A D K E T C F A E E G K K L V A A S Q 580

1741 GCT GCC TTA GGC TTA TAA CAT CTA CAT TTA AAA GCA TCT CAG 1782
581 A A L G L * 585

Figure 15D